**Migration Letters**

Volume: 21, No: S5 (2024), pp. 111-126

ISSN: 1741-8984 (Print) ISSN: 1741-8992 (Online)

www.migrationletters.com

# Green Synthesis Of Antibacterial Silver Nanoparticles Using Grewia Asiatica Leaf Extract: Characterization And Potential For Combatting Antibiotic-Resistant Bacteria

Abbas Ahmad[1], Javeed Muhammad[1], Khuzin Dinislam[2], Makarevich Maria[3], Voskresenskaya Elizaveta[3], Dzhukkayeva Madina[3], Orlov Alexey[4], Aydekova Khadizhat[3], Strok Polina[3], Oshurkova Ekaterina[5], Pimenova Victoria[5]

## Abstract

***Background:*** *Grewia asiatica Linn. is a shrub or small tree with a fruit of 5–12 mm diameter, having a purple to black color when it is ripe. The current global rise in antibiotic-resistant bacteria, associated with a steady decline in the development of new antibiotics has serious repercussions.*

***Objective:*** *The study aimed to develop and establish a safe, eco-friendly, cheaper method for the synthesis of silver nanoparticles using leaf extract of Grewia asiatica.*

***Methods:*** *The AgNPs were characterized using standard techniques, and their antimicrobial activity was evaluated against Acinetobacter baumannii and Salmonella enterica serovar Typhi to determine minimum inhibitory concentration.*

***Results:*** *The silver nanoparticles exhibit significant antibacterial activity against Acinetobacter baumannii and Salmonella typhi, their zone of inhibition was 8.43±1.53 nm and 8.34±1.73 nm, respectively. The XRD result revealed that the strongest reflection at 38.13º is the crystalline nature and face-centered-cubic structure of synthesized Ag-NPs. The green synthesis of three different AgNPs in different concentrations according to 0.1 molar, 0.01, and 1 molar solution was obtained to be 96 nm, 110nm, and 91nm in size respectively All samples have a low polydispersity index (PDI) of 0.46, 054, and 0.35, and their Zeta potential value was 23.7mv. FTIR spectrum result showed the presence of alkenes, Aromatic ketone, and alkyl halides which help in the reduction of silver ions to silver nanoparticles. The morphology of biosynthesized silver NPs further analyzed by scanning SEM the average size of AgNPs was found to be in the array of 40 nm to 80 nm. The analysis of optical properties of nanomaterials through Ultraviolet photo[1] spectrometry range between 200nm to 800nm revealed that AgNPs synthesized nanomaterial was seeing a peak of 477nm. Silver nanoparticles and leaf extract were tested against two-gram negative bacteria Acinetobacter baumannii and Salmonella*

[1]Department of Microbiology, University of Haripur, Haripur, Pakistan.
[2*]Department of Chemistry, Bashkir State Medical University, Russia.
[3]First Pavlov State Medical University of St. Petersburg, Russia.
[4]Moscow State Medical and Dental University, Russia.
[5]Ivanovo State Medical Academy Kate, Russia.
*Correspondence Khuzin Dinislam
[2*]Department of Chemistry, Bashkir State Medical University, Russia.

*typhi. AgNPs and G. asiatica extract indicate that Salmonella typhi is more sensitive to AgNPs and G. asiatica extract as compared to Acinetobacter baumannii.*

***Conclusion:*** *This study concludes that the green synthesis of silver nanoparticles by using Grewia asiatica plant extract is possible and these silver nanoparticles have potent antibacterial activity and can be used as an alternative to antibiotics. So, these nanoparticles can be used against multi-drug-resistant bacterial infections.*

**Keywords:** Green synthesis, Zeta potential, FTIR spectrum, Silver Nanoparticle, SEM Antibacterial activity

**Introduction:**

The current global rise in antibiotic-resistant bacteria, along with a negative trend in the development of new antibiotics, has major consequences[1]. Resistant bacteria drastically lower the chances of adequately treating infectious diseases and increase the risk of complications and death in individuals with blood infections. [2]. In the last two decades, antibiotic resistance has grown among numerous bacterial diseases. While this has long been known and a source of concern among infectious disease experts, prescribing physicians have only recently become aware of the issue. [3].Antimicrobial resistance increases the morbidity, mortality and costs of treating infectious diseases. [4]. Antibiotic resistance is becoming a greater issue nowadays because of irrational antibiotic use. As a result, selected pathogenic microorganisms that are resistant to numerous medications have proliferated [5]

Grewia asiatica Linn. (G. asiatica) family Malvaceae is a shrub or small tree with the fruit of 5– 12 mm diameter, having a purple to black color when it is ripe [8]. It is cultivated primarily for its edible fruit and is well-reputed for its diverse medicinal uses. G. Asiatica is considered to be native to the Indian subcontinent and Southeast Asia including Pakistan, and it is also commercially produced in the central parts of India and, the western northern region according to genealogical research conducted on this plant[9]. The chemical composition of G. asiatica contains the nutrient contents as well as various active metabolites such as tannins, phenols, flavonoids, alkaloids, triterpenoids, and steroids, as well as lignans, lactones, flavones, and anthocyanins [10], [11]. Therapeutic properties of G. asiatica are antidiabetic[12], hypoglycemic[13], radioprotective[14], antioxidant [12], hepatoprotective [15], antibacterial [16], analgesic, antiviral [17] and antipyretic[18] properties. It is also used in traditional Chinese medicine [17].

Nanotechnology is a novel research field of material science; it gains universal attention due to its broad-spectrum applications [19]. Nanotechnology is an interdisciplinary research area that imparts a wide range of applications in biology, physics, chemistry, biochemistry, engineering, and medicine in producing and using nanoparticle [20]. The real push has been growing new materials and looking at their properties by changing the molecule size and shape [21]. The particles with size 01nm-50nm, 50nm-100nm and 100nm-2500nm are referred to as nanoparticles (NPs), ultrafine nanoparticles, and fine nanoparticles respectively, are part of nanotechnology [22]. NPs completely show specific properties and characteristics such as unique size, geometry, distribution and morphology [23]. There are many ways to synthesize nanoparticles, including chemical, physical, and biological processes[24]. Each of these methods has a unique mix of benefits and drawbacks depending on the intended application. For instance, following synthesis, chemically produced nanoparticles (NPs) may be made available for functional testing extremely and quickly. Contrarily, NPs synthesized chemically have a variety of potential risks, including as cytotoxicity[25], genotoxicity[26], and carcinogenicity[27]. Physical procedure, on the other hand, are believed to take longer and are only possible under specific circumstances, such as higher temperatures or pressures, which

also increases the cost of the treatment[28]. Although most of the methods are successful in producing pure and well-defined nanoparticles, they are quite expensive or potentially dangerous to the environment[29]. Among various methods of synthesis of nanoparticles, the biological approach has been proved to be cost-effective, environment friendly are can be easily scaled up for large-scale synthesis[30]. However, past studies have demonstrated that microorganism-based nanoparticle synthesis is slower than plant-based (green) synthesis. When it comes to reducing metallic ions and making stable metal nanoparticles, plant extracts are better at it than microbes[31].

The aims and objective of this study to develop and establish safe, ecofriendly, cheaper method for the synthesis of silver nanoparticles using leaf extract of Grewia asiatica. After that verify and characterize synthesized silver nanoparticles with various analytical methods i.e., UV spectroscopy, FTIR, XRD, Zeta Sizer, SEM. And also evaluate antibacterial potential of the green synthesized silver nanoparticles against Acinetobacter baumannii and Salmonella typhi.

**Materials and Method:**

**Plant collection and study collaboration**

The research was carried out at the microbiology lab department of microbiology at The University of Haripur and the Public Health laboratory division at the National Institute of Health Islamabad. In October 2021, the Grewia asiatica plant was collected from the Cholistan Desert in the Bahawalpur area. The Botany department at the Islamia University of Bahawalpur did the identification and verification. "Ref. No. 154/botany" describes the Grewia asiatica plant (leaves) and the voucher. The dried leaves of Grewia asiatica were ground into powder using a grinder, and 400 grams of powder were employed for the entire study. They were then sieved and pulverized through mesh number 60 before being stored for further study. The crushed plant material was immersed in an aqueous methanolic solvent (30/70 v/v) for 5 days, with periodic stirring. It was then filtered through filter paper and muslin fabric. The extract was filtered, then evaporated at decreased pressure and between 30 and 40 degrees in a rotary evaporator. The outcome was a thick semisolid gummy type extract, which was then freeze-dried for collection of extract in powder form using the sublimation process. Once powder form was obtained, it was employed to synthesize silver nanoparticles shown in the Figure.1 [32].

**Figure 1: Green synthesis of silver Nanoparticles**

**Green synthesis of silver Nanoparticles**

Grewia asiatica extract acted as a reducing and stabilizing agent for Ag-NPs synthesis from silver nitrate. In a 0.1 Molar solution, 0.85 grams of AgNO3 were dissolved in 50 mL of distilled water. After adding 50 mg of the plant extract to another 50 mL of water, the extract solution was dropwise added to the silver nitrate solution with continuous stirring for 3 hours [33]. In a fresh trial with a 0.1 molar solution, 90g of powdered plant extract dissolved in 50ml of hydro methanol solvent, followed by magnetic stirring at 35°C for one hour. After filtration, 0.85g of AgNO3 in 50ml of distilled water was mixed dropwise with the plant extract on a magnetic stirrer, resulting in a color change from yellowish-brown to dark brown, indicating nanoparticle formation. Each sample underwent centrifugation for 10 to 30 minutes at 6000 RPM, followed by methanol rinse and drying [33].

**Characterization of Nanoparticles**

**UV–Visible spectroscopic analysis:**

The reaction mixture was subjected to verification for the reduction of silver ions and the synthesis of AgNPs using ultraviolet-visible spectroscopy. This spectrometric analysis involved detecting a peak within the 400–500 nm range to confirm the synthesis of Ag-NPs. The instruments employed during this process had a wavelength range of 200 to 800 nm, and water served as the reference, with a cut-off value at 190 nm. [34].

**Fourier transform-infrared spectral (FTIR) analysis**:

The analytical technique known as FTIR (Fourier-Transform Infrared) provides high-resolution IR spectra for solid or liquid materials. It offers insights into the functional groupings present in samples. In the study, FTIR was employed to characterize the reducing and stabilizing functional groups within the compound of Grewia Asiatica plant extract. The standard procedure was followed for sample preparation, and the spectrum was recorded in the range of 4000 - 600 cm-1 in diffuse reflectance mode, operating at a resolution of 4 cm-1[35].

**X-ray diffraction (XRD) analysis:**

X-ray diffraction analysis was employed for the detection of crystal shape and size. A total of 100 mg of air-dried samples from each concentration of AgNPs was documented to conclude the size and crystal structure of the nanoparticles, using XRD spectroscopy [36].

**Scanning electron microscope (SEM) and Energy dispersive X-ray (EDX) spectroscopy analysis:**

In SEM, the surface of the sample was scanned using a high-energy electron beam. Similar to TEM, the samples were analyzed after drying, but before analysis, they were coated with a thin layer of gold or platinum. The surface of nanoparticles could be directly observed using SEM. While this method provides size information, it is primarily employed to investigate surface morphology [37].

**Sonication:**

If the size of nanoparticles exceeded the desired range, they were sonicated for a few minutes using a SONOZAP ultrasonic homogenizer operating at 25 kHz (54). The ultrasonic processor transformed direct current into high-frequency electrical energy at a rate of 25 kHz or 25,000 cycles per second, generating mechanical vibrations. These vibrations produced pressure waves, leading to extreme agitation. As a result of this agitation, larger particles were broken down into nanoparticles through a shearing process [38].

**Bacterial liberary**

All the bacterial samples were collected from the National Institute of Health Islamabad, Pakistan (Acinetobacter baumannii and Salmonella enteric serovar Typhi).

**Media Preparation and Bacterial Confirmation:**

MacConkey media was prepared by adding 38 grams of powder to one liter of distilled water, adjusting the pH to 7.0, and sterilizing in an autoclave. After confirming sterilization with a color-changing tape, the media were poured into Petri plates and allowed to solidify. Confirmation of bacterial samples was done through API and MALDI-TOF biochemical tests, providing identification codes for database lookup. The API test strip contained freeze-dried reagents and color markers, while MALDI-TOF utilized soft ionization techniques in biological mass spectrometry for microbial identification.

**Antibacterial activity of silver nanoprticles through agar diffusion assay:**

Ag-NPs were tested for their antibacterial effectiveness against Salmonella serovar Typhi and Acinetobacter baumannii using the disc diffusion method. The lowest inhibitory concentration (MIC) was determined in MH broth medium. The antibacterial efficacy of bio-synthesized AgNPs was assessed against these bacterial strains using the conventional agar diffusion method, following Clinical and Laboratory Standards Institute recommendations (CLSI-2021). Discs of nanoparticles were placed in Petri dishes, incubated for 24 hours at 37°C under aerobic conditions. Various silver nanoparticle dilutions were applied in MIC, defining the MIC as the concentration preventing visible bacterial growth [34]. Cultures were diluted to 10^5 CFU/mL in Mueller-Hinton broth, spread on Mueller-Hinton Agar, and treated with AgNPs (200 mg/mL). A 6 mm inhibition zone confirmed antibacterial activity after incubation at 37°C for 24 hours [39].

**Antibacterial activity of silver nanoparticles through micro broth dilution method:**

A. baumannii and Salmonella typhi samples were cultured on MacConkey agar for 24 hours at 37˚C. After adjusting and diluting, a 96-well microtiter plate was used for MIC. The first column served as the control, columns 2 to 12 were filled with Mueller Hinton broth, and the 11th and 12th columns monitored bacterial growth control and media sterility. After 24 hours at 37°C, the plate was visually examined for results, considering media sterility and bacterial growth control.

**Statistical Analysis**

Data were compiled in an Excel sheet. Nanoparticle characterization employed manufacturer software for Zeta Sizer, XRD, UV-Vis, and Scanning Electron Microscope. The Independent T-test was utilized to compare zones of inhibitions and minimum inhibitory concentrations, assessing the effects of various treatments and bacterial isolates. SPSS software version 23.0 was employed for data analysis, with a p-value of 0.05 considered significant.

**Results and Discussion:**

**Phytochemical analysis:**

**Table 1: G. asiatica hydroalcoholic leaves extract exhibited flavonoids, steroids, terpenoids, alkaloids, tannins, and glycosides**.

| Phytochemical constituents | Grewia asiatica extract |
|---|---|
| **Alkaloids** | + |
| **Carbohydrates** | - |
| **Glycosides** | + |

| | |
|---|---|
| **Steroids** | + |
| **Tannins** | + |
| **Proteins** | - |
| **Terpenes** | + |
| **Saponins** | + |
| **Phenols** | + |
| **Flavonoids** | + |

**Characterization of Green synthesized AgNPs:**

**Visual Observation**

**Figure 1 illustrates the color change of the AgNO3 solution, transitioning from vivid yellow to dark brown upon the addition of G. asiatica methanolic extract. This observable change serves as physical evidence of AgNPs synthesis as reported by [41].**

**UV–visible spectroscopy**

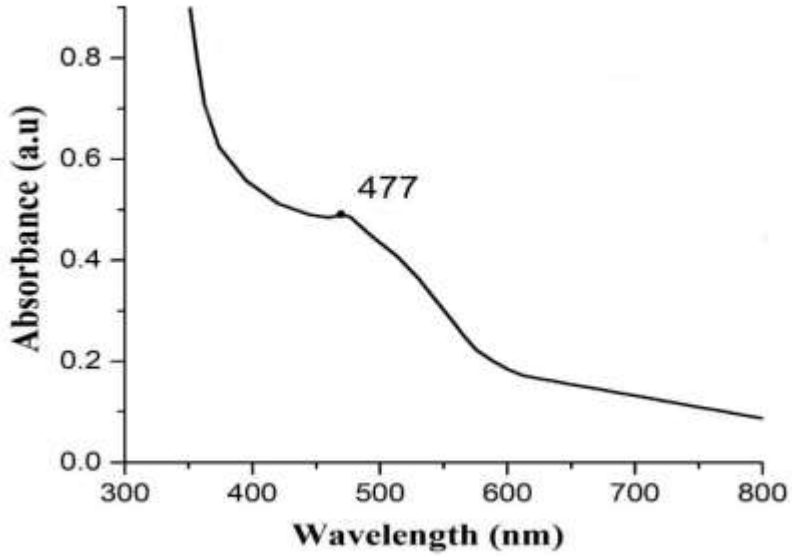
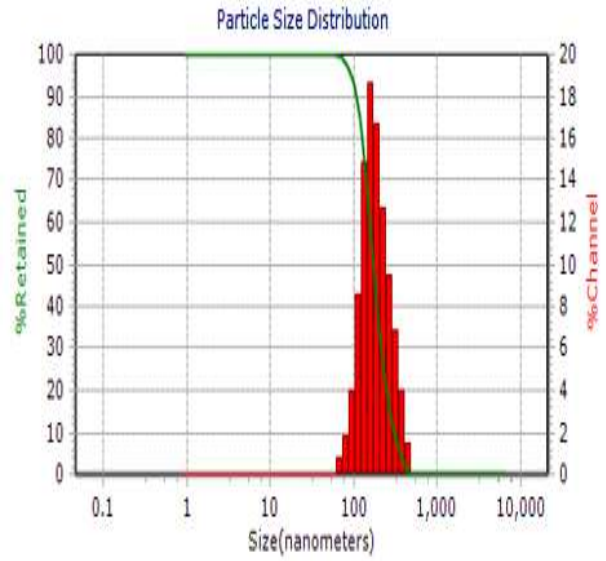


**Figure 2: displays spectroscopy, utilizing a peak in the range of 400-500nm to validate the production of AgNPs. The wavelength of the instruments used fell between 200-800nm, and Grewia Asiatica-mediated AgNPs exhibited a wavelength range of 435-477nm.**

**Zeta sizer (DLS) analysis**




| Measured Data | |
|---|---|
| Zeta Potential | 23.7 mv |
| Polarity | Positive |
| Mobility | 1.85u/s/V/cm |
| Conductivity | 112 uS/cm |
| Field Strength | 9.8 kV/m |
| SOP | |
| Zeta Run Time | 30 sec |

**Figure 3: shows the synthesis of three different AgNPs in various concentrations of G. asiatica, according to a 0.1 molar solution of silver nitrate. The sizes ranged from 80 nm to 500 nm. All samples exhibited a low polydispersity index (PDI) of 0.46, 0.54, and 0.35, with a Zeta potential value of 23.7mv.**

**X-ray diffraction (XRD) analysis**

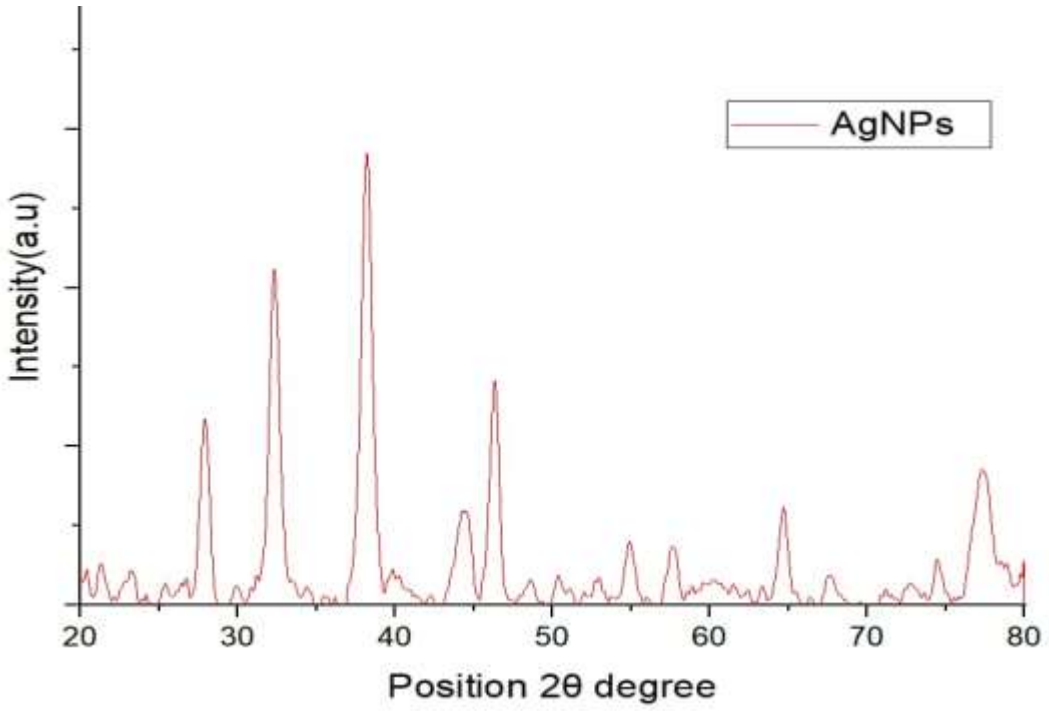



**Figure 4: Presents the XRD analysis of synthesized AgNPs using Grewia asiatica extract. Prominent diffraction peaks appear at 23.56º, 32.23º, 38.13°, 44.23°, 46.28º, 64.28°, and 77.438º, with the strongest diffraction observed at 38.13º.** (the planes indicate a face-centered cubic structure for the synthesized silver nanoparticles of varying sizes. The peak at 32.23°, though unassigned is presumed to be associated with the crystallization of additional organic compounds in Grewia asiatica extract). The results confirm the crystalline nature and face-centered cubic structure of AgNPs, with peak correlation to the reference card (JCPDS Card No. 4-0783) for synthesized AgNPs [42].

**FTIR Spectroscopy analysis**

**Table 2: The outcomes of FTIR analysis of this study give different stretches of the bond at altered peaks** (2981.40 cm-1, 92.27 % peaks indicate (C-H stretch); (C=0 stretch) of the Aromatic ketone. Peaks 1706.21 cm-1,94.16 % and 1599.31 cm-1,92.12% corresponded to N-H stretch. While 958.63 cm-1, 89.16% peaks indicate (N-H wag stretch); 591.56 cm-1,85.93%, 577.06cm-1, 563.99cm-1,557.92 cm-1 peaks specify (C-Br stretch).

| **Frequency RANGES (CM-1)** | **Functional group** |
|---|---|
| **3000-2850** | C-H stretching presence of alkenes |
| **1725-1650** | C=O stretching presences of Aromatic ketone |
| **1650-1550** | N-H stretching presences of Amine primary |
| **910-655** | N-H wag stretching presence of primary, secondary amines |
| **690-550** | C-Br stretching presence of alkyl halides |

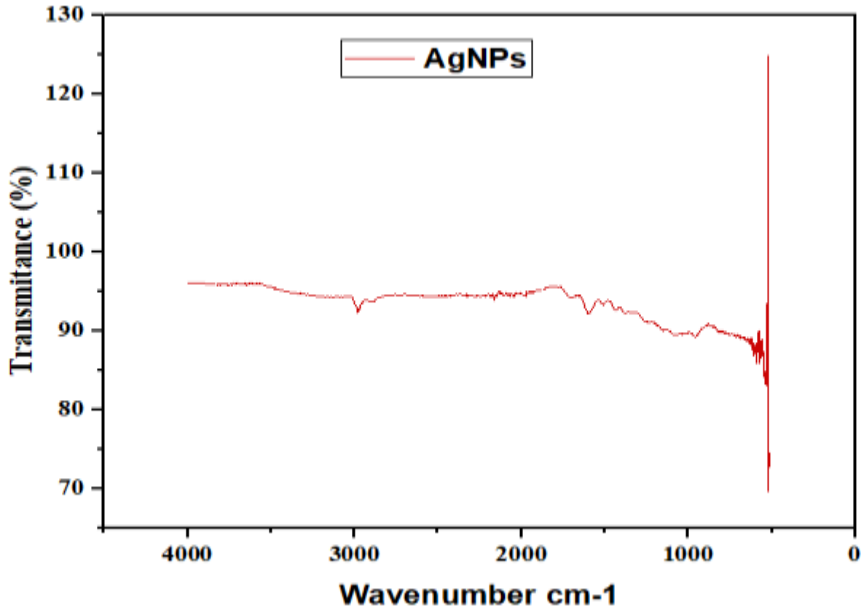



**Figure 5: The key phytochemical elements and bioactive compounds found in Grewia asiatica extract and nanoparticles were identified using FTIR spectroscopy.SEM analysis:**

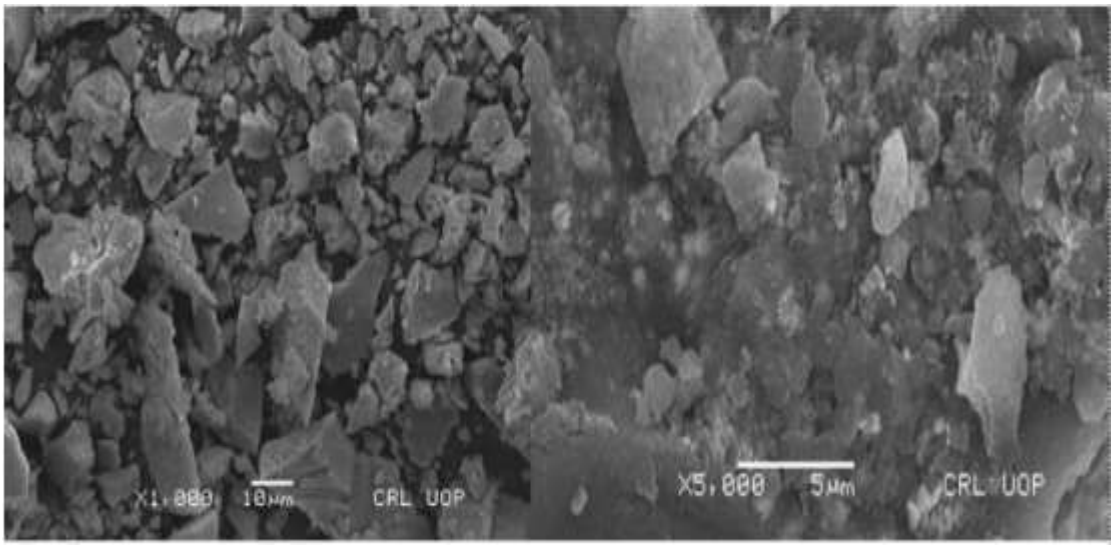



**Figure 6: SEM images of green synthesized Ag-NPs obtained by Grewia Asiatica extract at room temperature** (It was revealed that some silver nanoparticles were clumpy (cluster), and other were spherical. Some particles seem to be large, while others appear to be tiny in size. The morphology of the nanoparticles which vary in size from 72.7 to 239.1nm.

**Table: Comparison of zone of inhibition of G-AgNPs and G. asiatica Extracts agaisnt Salmonella typhi and Acinetobacter baumannii**

| Salmonella typhi | Acinetobacter baumannii |
|---|---|

| G-AgNPs Zone of Inhibition (mm) | G. asiatica Extracts Zone of Inhibition (mm) | G-AgNPs Zone of Inhibition (mm) | G. asiatica Extracts Zone of Inhibition (mm) |
|---|---|---|---|
| 10.1 | 6.2 | 11.4 | 6.9 |
| 10.0 | 8.1 | 10.6 | 7.2 |
| 10.1 | 6.4 | 9.9 | 8.0 |
| 11.0 | 7.5 | 9.7 | 6.2 |
| 8.9 | 7.0 | 9.7 | 7.0 |
| 9.9 | 7.4 | 9.4 | 7.7 |
| 9.5 | 7.0 | 8.3 | 7.4 |
| 10.7 | 6.7 | 10 | 8.3 |
| 7.3 | 5.5 | 9.2 | 7.8 |
| 10.4 | 7.1 | 8.5 | 5.5 |

**Table: Satistical Characteristics of zone of inhibition of G-AgNPs and G. asiatica Extracts among Salmonella typhi and Acinetobacter baumannii**

| Variables | Mean Zone of inhibition± SD | 95% CL of the Difference | | P value |
|---|---|---|---|---|
| | | Lower | Upper | |
| **G-AgNPs** | 9.73±0.96 | 2.12 | 3.24 | 0.00 |
| **G. asiatica Extracts** | 7.04±0.78 | | | |
| **Salmonella typhi** | 8.34±1.73 | 0.95 | 1.14 | 0.85 |
| **Acinetobacter baumannii** | 8.43±1.53 | | | |

**Discussion:**

The synthesis of three different AgNPs in different concentrations of Grewia asiatica extract according to 0.1 molar solution was found to be 80- 500nm in size respectively. All samples had a low polydispersity index (PDI) of 0.46, 054, and 0.35, and their Zeta potential value was 23.7mv. The particles size distribution was 80nm to 200nm.The size of the produced AgNPs' particles is determined using a technique called dynamic light scattering measurement. The biogenic AgNPs' size distribution is as seen the diameters of the particles were found to results were in the 50–300 nm range. The typical dimension of the Using the leaf extract from Salvadora persica, biogenic AgNPs are 153 nm or so [43]. By the (K Ahmad et al. 2020) they developed yields for silver nanoparticles of various sizes. Plot 1, with a molecule size of 92 nm and a zeta potential of 24.8 mV, was the optimal layout. [44]. The zeta potential of the cube-shaped nanoparticle formulation FC2 with particle size 505.3 nm was found to be -3.15 mV and PDI 0.588, whereas the zeta potential of the spherical nanoparticle formulation FS2 with particle size 523.7 nm was found to be 4.87 mV and PDI 0.599 [45].

The analysis of optical properties of nanomaterials was analyzed through Ultraviolet photo spectrometry. The spectroscopy uses a peak between 400-500nm to validate the production of AgNPs. The wavelength of the instruments used was between 200-800nm. Grewia asiatica-

mediated AgNPs have a wavelength of 435-477nm. The integration of nanoparticles UV apparent spectroscopy was carried out in a previous investigation. At 450 nm, where the green amalgamation of silver nanoparticles can be seen, the largest absorption band of silver nanoparticles was seen. [46]. According to [47] study, To determine the crystalline nature and structure of silver nanoparticles, an X-ray diffractogram (XRD) study of dried green produced AgNPs by Prunus persica extract was performed. Diffraction peaks at 2 thetas including 38.20, 44.23, 64.33, 77.40, 27.741, 32.221, 46.261, and 81.67 were visible in the pattern. The spectra displayed five distinct peaks that could be indexed to the (111), (200), (220), (311) and (222) reflection planes of the face-centered cubic structure of silver at 2h=38.20, 44.23, 64.33 and 77.40, respectively. [47].

The XRD patterns of synthesized AgNPs obtained by using Grewia asiatica extract are shown in Figure. There are different prominent diffraction peaks at, 23.56º, 32.23º, 38.13°, 44.23°, 46.28º, 64.28°, and 77.438º. The strongest reflection at 38.13º These planes represent a face-centered cubic structure of synthesized various-sized AgNPs. The Türkekul, İ. and Gökce, İ., 2021 study used a high-resolution SEM picture was examined to focus on the external shape of Ag NPs. The random, clumpy, and some circular type silver nanoparticles were seen in the SEM examination. Some molecule appears to be large and some appear little in size (Kaplan et al.,2021.). The average size of AgNPs was found to be in the array of 40 nm to 80 nm. On the other hand, SEM images showed particle average ssizes and morphology of the nanoparticles which vary in size from 72.7 to 239.1nm. respectively. The larger silver nanoparticles in SEM may be due to the agglomeration of smaller size AgNPs (A. M. set al., 2013).

Gram-negative bacteria were more affected by silver nanoparticles than Gram-positive bacteria. This may be because Gram-negative bacteria have relatively more negative charges than positive charges, which facilitates the interaction of the nanoparticles with the cell wall. [50]. Since silver is a soft acid, it will inevitably react with a soft base because an acid's natural propensity is to do so. Sulfur and phosphorus, two soft bases, make up the majority of the components of the cells. These nanoparticles' impact on the cell may trigger a process that ultimately results in cell death.[51].

According to [32] All of the silver nanoparticles' principal peaks could be seen in the FTIR spectrum. Peaks for the OH-group and C=O groups were seen at 3385.04 cm-1 and 1660.09 cm-1, respectively. The peak of the NH group was measured at 1596.20 cm -1. [52] The spectra displayed absorption peaks at 3313 cm-1, 1635 cm-1, 1070 cm-1, and 1032 cm, indicating the existence of chemicals that are biologically active. (Khalil Ahmad et al., 2021

The outcomes of FTIR analysis of this study give different stretches of bond at altered peaks; 2981.40 cm-1,92.27 % peaks indicate (C-H stretch); (C=0 stretch) of the Aromatic ketone at 1706.21 cm-1,94.16 %; peaks at 1599.31 cm-1,92.12% showed (N-H stretch); while 958.63 cm-1,89.16% peaks indicate (N-H wag stretch); 591.56 cm-1,85.93%, 577.06cm-1, 563.99cm-1,557.92 cm-1 peaks specify (C-Br stretch);

[53] 3.9 to 7.8 g/mL was the range of the MIC values for AgNPs against foodborne pathogens. The MIC values for K. pneumonia, S. Typhimurium, and S. Enteritidis were 3.9 g/mL, while the MIC value for E. coli was 7.8 g/mL. After 2 hours of incubation, AgNPs for E. coli reached their bactericidal endpoint at 4 MIC (31.2 g/mL) and 8 MIC (62.4 g/mL) When K. pneumonia was incubated at 2 MIC (7.8 g/mL), 4 MIC (15.6 g/mL), and 8 MIC (31.2 g/mL) for 2 hours, the germs were destroyed. After 1 hour of incubation at 4 MIC (15.6 g/mL) and 8 MIC (31.2 g/mL), S. Typhimurium was destroyed. After 2 hours of incubation at 2 MIC (7.8 g/mL) and 4 MIC (15.6 g/mL), the bactericidal endpoint of AgNP for S. Enteritidis was attained; however, the endpoint was reached more quickly after 1 hour at 8 MIC (31.2 g/mL).

Silver nanoparticles were tested against the same sample of Acinetobacter baumannii and Salmonella was tested. Our study showed In vitro susceptibility to AgNPs. out of 20 samples, 03 samples' MIC value was (0.78µg/mL), 04 sample susceptible to AgNPs (MIC 1.56µg/mL), 01 sample susceptible to AgNPs (MIC 6.25µg/mL) and 02 sample susceptible to AgNPs (MIC 3.12µg/mL). moreover, the percentage of extracted nanoparticles was different in some samples 03/10(30%), 4/10(40%), 1/10(10%), and 2/10(10%) respectively. where 04 samples salmonella typhi with MIC value (0.78µg/ml), 02 sample (MIC 1.56µg/mL), 03 isolated were investigated with MIC (3.12µg/mL and 01 was recorded (MIC6.25µg/mL the percentage of extracted nanoparticles was different in some samples 04/10(40%), 2/10(20%), 3/10(30%), and 1/10(10%) respectively.

**Conclusion:**

The current study uses Grewia asiatica extract to define the development of AgNPs. The reduction and capping of AgNO3 to silver nanoparticles are caused by the bioactive compounds present in G. asiatica leaves. The capping ingredient stabilizes the silver nanoparticles. Functional groups on the surface of Silver NPs are what cause this activity. Furthermore, due to their small size and the presence of capping agents, silver nanoparticles show long-lasting antibacterial activity against both bacteria. AgNPs have the potential to be employed as antibiotics in the future because they are highly powerful against bacteria, non-toxic, inexpensive, and environmentally benign. These findings imply that silver nanoparticles can effectively combat infections brought on by multidrug-resistant isolates, which pose a severe danger to global public health. Future research will focus on the possibility of broadening the use of the produced nanoparticles by adding effective antibiotics to their surfaces. These particles could aggressively and precisely eliminate microorganisms that were resistant to many antibiotics.

**References:**

[1] L. Serwecińska, "Antimicrobials and Antibiotic-Resistant Bacteria: A Risk to the Environment and to Public Health," Water, vol. 12, no. 12, p. 3313, Nov. 2020, doi: 10.3390/w12123313.

[2] O. Cars and P. Nordberg, "Antibiotic resistance-The faceless threat," International Journal of Risk & Safety in Medicine, vol. 17, pp. 103–110, 2005.

[3] R. Finch, "Bacterial resistance—the clinical challenge," Clinical Microbiology and Infection, vol. 8, no. SUPPL. 3, pp. 21–32, 2002, doi: 10.1046/J.1469-0691.8.S.3.3.X.

[4] P. M. Hawkey and A. M. Jones, "The changing epidemiology of resistance," Journal of Antimicrobial Chemotherapy, vol. 64, no. suppl_1, pp. i3–i10, Sep. 2009, doi: 10.1093/JAC/DKP256.

[5] F. C. Tenover, "Mechanisms of Antimicrobial Resistance in Bacteria," The American Journal of Medicine, vol. 119, no. 6, pp. S3–S10, Jun. 2006, doi: 10.1016/J.AMJMED.2006.03.011.

[6] P. Mittal, R. Bisht, A. Katiyar, and R. Singh, "P. Mittal, V. Juyal. Drug-dietary interaction potential of garlic on glimepiride treated type 2 diabetic wistar rats Patient Safety in OTC products View project Evaluation of interaction potential of glimepiride with some dietary supplements View project ," Asian Journal of Pharmaceutical and Clinical Research, vol. 3, no. 2, 2012.

[7] N. F.-M. del I. de I. en Ciencias and undefined 2016, "Bacterial resistance: A global public health problem with difficult solution," scielo.iics.una.py.

[8] M. Zia-Ul-Haq, M. S. Stanković, K. Rizwan, and V. De Feo, "Grewia asiatica L., a food plant with multiple uses," Molecules, vol. 18, no. 3, pp. 2663–2682, Mar. 2013, doi: 10.3390/MOLECULES18032663.

[9] S. Halder et al., “Herbal drugs and natural bioactive products as potential therapeutics: A review on pro-cognitives and brain boosters perspectives.,” Saudi pharmaceutical journal : SPJ : the official publication of the Saudi Pharmaceutical Society, vol. 29, no. 8, pp. 879–907, Aug. 2021, doi: 10.1016/j.jsps.2021.07.003.

[10] P. Patil, M. M. Patel, and C. J. Bhavsar, “Preliminary phytochemical and hypoglycemic activity of leaves of Grewia Asiatica L.,” Research Journal of Pharmaceutical, Biological and Chemical Sciences, vol. 2, no. 1, pp. 516–520, 2011.

[11] W. Ullah, G. Uddin, and B. S. Siddiqui, “Ethnic uses, pharmacological and phytochemical profile of genus Grewia,” http://dx.doi.org/10.1080/10286020.2011.639764, vol. 14, no. 2, pp. 186–195, Feb. 2012, doi: 10.1080/10286020.2011.639764.

[12] N. A. Khatune, B. M. Rahman, R. K. Barman, and M. I. I. Wahed, “Antidiabetic, antihyperlipidemic and antioxidant properties of ethanol extract of Grewia asiatica Linn. bark in alloxan-induced diabetic rats,” BMC Complementary and Alternative Medicine, vol. 16, no. 1, Aug. 2016, doi: 10.1186/S12906-016-1276-9.

[13] M. A. Mesaik et al., “Effect of Grewia asiatica fruit on glycemic index and phagocytosis tested in healthy human subjects,” Pakistan Journal of Pharmaceutical Sciences, vol. 26, no. 1, pp. 85–89, Jan. 2013.

[14] K. V. Sharma and R. Sisodia, “Evaluation of the free radical scavenging activity and radioprotective efficacy of Grewia asiatica fruit,” Journal of radiological protection : official journal of the Society for Radiological Protection, vol. 29, no. 3, pp. 429–443, 2009, doi: 10.1088/0952-4746/29/3/007.

[15] K. V Sharma, R. Sisodia, and K. V Sharma, “Hepatoprotective efficacy of Grewia asiatica fruit against oxidative stress in swiss albino mice,” 2010, Accessed: Apr. 16, 2022. [Online]. Available: https://www.sid.ir/en/Journal/ViewPaper.aspx?ID=180901.

[16] A. Tahir et al., “Green synthesis, characterization and antibacterial, antifungal, larvicidal and anti-termite activities of copper nanoparticles derived from Grewia asiatica L.,” Bulletin of the National Research Centre, vol. 46, no. 1, Dec. 2022, doi: 10.1186/S42269-022-00877-Y.

[17] S. Jyoti, P. Shalini, K. C. Satya, and R. Gyanendra, “Nutritional and medicinal potential of Grewia subinaequalis DC. (syn. G. asiatica.) (Phalsa),” Journal of Medicinal Plants Research, vol. 9, no. 19, pp. 594–612, 2015, doi: 10.5897/jmpr2015.5724.

[18] B. Akhtar et al., “Analgesic, antipyretic and anti-inflammatory activities of Grewia asiatica fruit extracts in albino mice,” Acta Poloniae Pharmaceutica - Drug Research, vol. 73, no. 4, pp. 983–989, Jul. 2016.

[19] S. Nasr-Eldahan, A. Nabil-Adam, M. A. Shreadah, A. M. Maher, and T. El-Sayed Ali, “A review article on nanotechnology in aquaculture sustainability as a novel tool in fish disease control,” Aquaculture International, vol. 29, no. 4, pp. 1459–1480, Aug. 2021, doi: 10.1007/S10499-021-00677-7.

[20] A. B.-A. S. N. Health and undefined 2020, “Nanomaterials in food processing and packaging, its toxicity and food labeling,” academia.edu, 2020, Accessed: Aug. 18, 2022. [Online]. Available: https://www.academia.edu/download/64280219/ASNH-04-0745.pdf.

[21] A. Pearce, T. Wilks, M. A.-N. R. Chemistry, and undefined 2021, “Synthesis and applications of anisotropic nanoparticles with precisely defined dimensions,” nature.com, Accessed: Aug. 18, 2022. [Online]. Available: https://www.nature.com/articles/s41570-020-00232-7.

[22] B. Hafiz Saad Ahmad, “Biogenic Synthesis and Characterization of Silver Nano Particles Using ‘Dipterygium glaucum’ Extract and its AntiCancer Potential Against Various Cancer Cell Lines.,” 2020, Accessed: Aug. 18, 2022. [Online]. Available: http://itr.iub.edu.pk:8000/xmlui/handle/123456789/1239.

[23] M. A. Shenashen, S. A. El-Safty, E. A. Elshehy M A Shenashen, S. A. El-Safty, and E. A. Elshehy, "Synthesis, morphological control, and properties of silver nanoparticles in potential applications," Wiley Online Library, vol. 31, no. 3, pp. 293–316, 2013, doi: 10.1002/ppsc.201300181.

[24] S. Iravani, … H. K.-R. in, and undefined 2014, "Synthesis of silver nanoparticles: chemical, physical and biological methods," ncbi.nlm.nih.gov, Accessed: Aug. 18, 2022. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4326978/.

[25] N. Lewinski, V. Colvin, R. D.- small, and undefined 2008, "Cytotoxicity of nanoparticles," Wiley Online Library, vol. 4, no. 1, pp. 26–49, Jan. 2008, doi: 10.1002/smll.200700595.

[26] H. Xie, M. M. Mason, and J. P. Wise, "Genotoxicity of metal nanoparticles," Reviews on Environmental Health, vol. 26, no. 4, pp. 251–268, Dec. 2011, doi: 10.1515/REVEH.2011.033/HTML.

[27] L. Liu, L. K.-J. of A. Toxicology, and undefined 2021, "Research progress on the carcinogenicity of metal nanomaterials," Wiley Online Library, vol. 41, no. 9, pp. 1334–1344, Sep. 2021, doi: 10.1002/jat.4145.

[28] E. K. Schneider-Futschik and F. Reyes-Ortega, "Advantages and Disadvantages of Using Magnetic Nanoparticles for the Treatment of Complicated Ocular Disorders.," Pharmaceutics, vol. 13, no. 8, Jul. 2021, doi: 10.3390/pharmaceutics13081157.

[29] A. A. Yaqoob, K. Umar, and M. N. M. Ibrahim, "Silver nanoparticles: various methods of synthesis, size affecting factors and their potential applications–a review," Applied Nanoscience (Switzerland), vol. 10, no. 5, pp. 1369–1378, May 2020, doi: 10.1007/S13204-020-01318-W.

[30] M. Ramya, M. S.-I. J. P. M. B. Sci, and undefined 2012, "Green synthesis of silver nanoparticles," Citeseer, 2012, Accessed: Aug. 18, 2022. [Online]. Available: http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.300.5846&rep=rep1&type=pdf.

[31] P. Mondal, A. Anweshan, M. P.- Chemosphere, and undefined 2020, "Green synthesis and environmental application of iron-based nanomaterials and nanocomposite: a review," Elsevier, Accessed: Aug. 18, 2022. [Online]. Available: https://www.sciencedirect.com/science/article/pii/S0045653520317033?casa_token=UQClv_h-FjwAAAAA:9BsXekOvtnKnUsMjCHB0Jho_rpl5aUgL6HY9ILX0Ri72mt8sHloQKoGLDQ-bTIVAagZzMHj6Zk0.

[32] K. Ahmad et al., "Green Synthesis and Characterization of Silver Nanoparticles using Crude Extract of Crotalaria burhia," RADS Journal of Pharmacy and Pharmaceutical Sciences, vol. 8, no. 4, pp. 1–7, Mar. 2021, doi: 10.37962/jpps.v8i4.464.

[33] M. Ovais et al., "Green synthesis of silver nanoparticles via plant extracts: Beginning a new era in cancer theranostics," Nanomedicine, vol. 12, no. 23, pp. 3157–3177, Dec. 2016, doi: 10.2217/NNM-2016-0279.

[34] M. Singh, S. Singh, S. Prasad, I. G.-D. J. of, and undefined 2008, "Nanotechnology in medicine and antibacterial effect of silver nanoparticles," maaz.ihmc.us, vol. 3, no. 3, pp. 115–122, 2008.

[35] M. A. Sabri, A. Umer, G. Hameed Awan, M. F. Hassan, and A. Hasnain, "Selection of suitable biological method for the synthesis of silver nanoparticles," journals.sagepub.com, vol. 6, no. 1, 2016, doi: 10.5772/62644.

[36] S. Ahmed, M. Ahmad, B. Swami, S. I.-J. of advanced research, and undefined 2016, "A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise," Elsevier.

[37] A. Awwad, N. S.-N. and Nanotechnology, and undefined 2012, "Green synthesis of silver nanoparticles byMulberry LeavesExtract," eacademic.ju.edu.jo, vol. 2, no. 4, pp. 125–128, 2012,

doi: 10.5923/j.nn.20120204.06.

[38] T. Sreekanth, P. Nagajyothi, … P. M.-… of P. and, and undefined 2018, "Ultra-sonication-assisted silver nanoparticles using Panax ginseng root extract and their anti-cancer and antiviral activities," Elsevier.

[39] K. Narayanan, N. S.-A. in colloid and interface science, and undefined 2010, "Biological synthesis of metal nanoparticles by microbes," Elsevier.

[40] J. Singh, T. Dutta, K. H. Kim, M. Rawat, P. Samddar, and P. Kumar, "'Green' synthesis of metals and their oxide nanoparticles: Applications for environmental remediation," Journal of Nanobiotechnology, vol. 16, no. 1, Oct. 2018, doi: 10.1186/S12951-018-0408-4.

[41] A. Saxena Envisics, R. M. Tripathi, A. Saxena, R. M. Tripathi, F. Zafar, and P. Singh, "Green synthesis of silver nanoparticles using aqueous solution of Ficus benghalensis leaf extract and characterization of their antibacterial activity," Elsevier, vol. 67, 2011, doi: 10.1016/j.matlet.2011.09.038.

[42] D. Bharathi, M. Diviya Josebin, S. Vasantharaj, and V. Bhuvaneshwari, "Biosynthesis of silver nanoparticles using stem bark extracts of Diospyros montana and their antioxidant and antibacterial activities," Journal of Nanostructure in Chemistry, vol. 8, no. 1, pp. 83–92, Apr. 2018, doi: 10.1007/S40097-018-0256-7.

[43] N. A. Alshaye, M. M. Elobeid, D. H.M. Alkha, and A. E. Mohamme, "Characterization of Biogenic Silver Nanoparticles by Salvadora persica Leaves Extract and its Application Against Some MDR Pathogens E. coli and S. aureus," Research Journal of Microbiology, vol. 12, no. 1, pp. 74–81, 2016, doi: 10.3923/jm.2017.74.81.

[44] K. Ahmad et al., "Green Synthesis and Characterization of Silver Nanoparticles using Crude Extract of Crotalaria burhia," jpps.juw.edu.pk, vol. 8, no. 4, pp. 209–215, 2020.

[45] G. Raghvendra, N. Kumar Pandey, B. Kumar, S. Singh, P. Sharma, and Y. Sarvi, "Investigation of influence of shape on drug loading and entrapment efficiency of nanoparticles," Journal of Pharmacy Research, vol. 11, no. 7, pp. 850–855, 2017.

[46] M. Khan, F. Tareq, … M. H.-J. of E., and undefined 2018, "Green synthesis and characterization of silver nanoparticles using Coriandrum sativum leaf extract," jestec.taylors.edu.my, vol. 13, no. 1, pp. 158–166, 2018.

[47] R. Rajkumar, & N. S.-J. of N., and undefined 2015, "To Study the Effect of the Concentration of Carbon on Ultraviolet and Visible Light Photo Catalytic Activity and Characterization of Carbon Doped TiO^ sub 2^," researchgate.net.

[48] Ö. Kaplan, N. Tosun, A. Özgür, … S. T.-J. of D. D., and undefined 2021, "Microwave-assisted green synthesis of silver nanoparticles using crude extracts of Boletus edulis and Coriolus versicolor: Characterization, anticancer, antimicrobial," Elsevier.

[49] A. M. Awwad, N. M. Salem, and A. O. Abdeen, "Green synthesis of silver nanoparticles using carob leaf extract and its antibacterial activity," International Journal of Industrial Chemistry, vol. 4, no. 1, p. 29, 2013, doi: 10.1186/2228-5547-4-29.

[50] B. Sadeghi, M. Jamali, S. Kia, A. Amini Nia, and S. Ghafari, "Synthesis and characterization of silver nanoparticles for antibacterial activity," Int.J.Nano.Dim, vol. 1, no. 2, pp. 119–124, 2010.

[51] S. Prabhu and E. K. Poulose, "Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects," International Nano Letters, vol. 2, no. 1, Dec. 2012, doi: 10.1186/2228-5326-2-32.

[52] K. R, G. G, J. A, and G. M, "Rapid Green Synthesis of Silver Nanoparticles (AgNPs) Using (Prunus persica) Plants extract: Exploring its Antimicrobial and Catalytic Activities," Journal of

Nanomedicine & Nanotechnology, vol. 08, no. 04, 2017, doi: 10.4172/2157-7439.1000452.

[53] Y. Y. Loo et al., "In Vitro antimicrobial activity of green synthesized silver nanoparticles against selected Gram-negative foodborne pathogens," Frontiers in Microbiology, vol. 9, no. JUL, pp. 1–7, 2018, doi: 10.3389/fmicb.2018.01555.